Acute Combination—Pharmacological and Thrombolytic and Chronic Neurorestorative Treatment of Experimental Stroke

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This presentation will be divided into two main sections. In the first (A), we will provide data on the temporal profiles of changes occurring acutely within the first hours and days after the onset of embolic stroke. We will describe the microvascular changes that occur after onset of stroke that contribute to the progression and maturation of ischemic cell damage and blood brain barrier disruption. Therapeutic interventions designed to extend the therapeutic window for thrombolysis and the reduction of adverse effects, like hemorrhagic transformation will be discussed. Combination therapies will feature prominently in this presentation. MRI will be shown to provide insight into perfusion and vascular changes that reflect the pathophysiological events after stroke and the effects of the acute vascular and neuroprotective therapeutic interventions.

The second part of this lecture will focus on neurorestorative approaches to the treatment of stroke and the role of MRI in contributing to the development of these therapies, in terms of monitoring the effects of therapy on tissue and identifying and predicting therapeutic benefit.

A. In all our studies which involve MRI and thrombolysis we employ an embolic model of stroke in the rat. A fibrin rich 24 hour old engineered clot is placed at the origin of the middle cerebral artery (MCA). This clot is often retained at the site for at least 24 hours. The frequency and temporal profiles of autolysis and hemorrhage, both petechial and gross hemorrhage are consistent with that found in the human. The model mimics the human condition in the response of the rodent to thrombolysis with recombinant tissue plasminogen activator inhibitor (rtPa). Rats subjected to embolic stroke and treated within a 3 hour time window after stroke show a reduction in the volume of cerebral infarction and significant functional improvement. Rats treated beyond this 3 hour window show no therapeutic benefit.

   We have employed this model of embolic stroke to investigate the temporal profiles of change in the relationship of MCA patency to microvascular perfusion, BBB disruption, and we have performed an array of MRI measurements, including ADC, Ki, T1, T1sat, T2, SWI and others, in documenting the temporal profiles of change after embolic stroke. We will also demonstrate that a cluster analysis approach to which integrates information from a set of MRI parameters (e.g. ISODATA) provides a highly sensitive method to characterize cerebral tissue damage, providing a non-invasive grading of the degree of ischemic cell damage. We have performed this multiple parameter ISODATA using image intensities and maps of MRI parameters. Comparisons of these approaches will be presented. In addition, data on the ability of MRI to identify BBB disruption and the relationship of this BBB disruption to the development of parenchymal bleed will be described. In general, our data demonstrate that sets of MRI parameters and maps can predict hemorrhagic transformation as well as characterize ischemic cell damage.

We will also describe our approach to treat ischemic stroke. Acutely after the onset of stroke, a tapestry of events are woven which can extend and increase the severity of ischemic cell damage. These events include the activation of inflammatory cells, e.g. platelets, neutrophils, matrix metalloproteinases, integrins, adhesion molecules and the creation of a procoagulation state within the microvasculature which exacerbates ischemic cell damage. These events which are time dependent occur both at the site of occlusion at the origin of the MCA as well as downstream within the cerebral microvasculature. Treatment of stroke with a thrombolytic agent such as the one in clinical use, recombinant tissue plasminogen activator (rtPa), can exacerbate these events. Although the rtPa can lyse the clot, which is a necessary condition for salvaging the cerebral tissue, adverse effects, associated with the use of rtPa counter some of the benefit potentially derived from the treatment. These effects are amplified at 4 or more hours after the onset of stroke. Thus agents which can neutralize of reduce some of these interlaced events resulting from the use of a thrombolytic agent, may provide therapeutic benefit. We show that the therapeutic window for rtPa can be significantly extended beyond the 3 hours and the hemorrhagic transformation resulting from the use of rtPa can be reduced. Our laboratory has tested this hypothesis using an array of agents which reduce inflammation, integrins, adhesion molecules and others.

In this presentation, we will focus on the use of statins, GPIIB/IIIA receptor antagonists and proteosome inhibitors in the combination treatment of stroke with rtPa. Briefly, to focus on one particular agent, statins will be selected. Statins are drugs designed to reduce cholesterol, they are HMG-CoA reductase inhibitors which reduce total cholesterol and LDL. These agents are also highly pleiotropic, and have potent anti inflammatory and anticoagulation effects. They reduce the increase in protease activated receptor 1 (PAR-1), adhesion molecules such as ICAM-1, matrix metalloproteases, and upregulate molecules which maintain the integrity of the BBB. We have tested atorvastatin in combination with rtPa for the treatment of embolic stroke with treatment initiated at 4 hours after stroke onset. Histology, and single cell laser capture microscopy as well as MRI were performed on tissue and the living animal with and without treatment. Our data reveal that statins, which are relatively benign drugs, have significantly extended the therapeutic window for rtPa and enhance the integrity of the BBB. These agents which are in common clinical use can be readily employed for the treatment of acute stroke. Additional data on a GPIIb/IIA receptor antagonist, Abciximab (ReoPro) when employed in combination with rtPa can also extend the therapeutic window for stroke. Similar data will be presented with a proteosome inhibitor.

B. The second part of this talk will focus on neurorestorative therapies. We have shown that both cell-based and pharmacologically based therapies initiated at not less than 1 day post stroke have remarkable therapeutic potential to significantly reduce neurological deficits resulting from stroke. Our data support the hypothesis that these treatments “remodel” brain. They induce angiogenesis, neurogenesis, and synaptogenesis and reduce the thickness and intensity of glial scarring. Data will be presented on the use of cell based therapies. We will focus primarily on mesenchymal cells derived from the bone marrow and progenitor and stem cells derived from the subventricular zone in the adult rodent brain. These cells activate the endogenous parenchymal cells to induce a variety of neurotrophic factors which amplify neuro-remodeling events in brain, which subsequently lead to improved neurological function after stroke. We have also employed other cells, including cord blood cells and fetal cells as therapeutic cell based approaches for the treatment of neurological diseases. In addition, we will discuss the use of pharmacologic therapies which complement and independently promote improved neurological outcomes

after stoke. We will focus on agents who increase cyclic guanosine monophosphate (e.g. sildenafil), statins and erythropoietin. All these drugs presently employed clinically for the treatment of other diseases have the remarkable ability to enhance neurological recovery after stroke, traumatic brain injury and neurodegenerative disease, e.g. EAE-multiple sclerosis. The preclinical data for both restorative treatment of stroke and TBI will be provided. In addition, our application of MRI in the development of these therapies, in the monitoring of treatment and in the evaluation of therapeutic response will be provided. Our group was the first to employ adult cell labeling using ferrite nanoparticles to monitor the movement of cells injected into the cisterna magna after the onset of stroke. We will present data on using MRI to evaluate the response of the injured brain to the neurorestorative treatment. A primary hypothesis is that neurorestorative treatment enhances angiogenesis and neurogenesis. We will provide data that angiogenesis can be monitored and predicted after the use of cell and pharmacologically based therapies. Neurogenesis, white matter and axonal and dendritic changes plasticity from the neurorestorative cell or pharmacologically based treatment may be visualized using MRI. We propose and we will provide data to support the hypothesis that after stroke and treatment, white matter structure is altered which reflects these neurorestorative processes.

In summary, ischemic stroke should be treated using a bi-directional approach. Acutely interventions are needed which increase tissue perfusion and inhibit adverse sequellae of stroke and treatment with rtPa. Subsequent to the acute vascular and neuroprotective treatment, neurorestorative therapy will be invoked. These treatments, either cell or pharmacological can be initiated chronically (e.g. 1 month) after stroke. Brain plasticity is enhanced, glial scar is reduced, angiogenesis and neurogenesis are amplified, all of which contribute to the reduction of neurological deficit. MRI plays an important role in the development of these therapies as well as to their application to living systems.